Honeydew producers in eucalypts and associated native fauna

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Abstract

In recent years there has been an increase in the number of introduced eucalypt-feeding invasive species in worldwide plantations outside Australia. A large portion of these are honeydew producing sap-suckers, mostly psyllids (Hemiptera: Psylloidea). Besides the negative impact on eucalypt production, these non-native honeydew producers may have ecological effects on local native fauna by establishing new interactions. In this work, we intended to study these new interactions in eucalypt plantations in Portugal.

We surveyed during a year three invasive psyllid species, *Glycaspis brimblecombei*, *Ctenarytaina spatulata* and *Ctenarytaina eucalypti*, in two sites with eucalyptus trees in Lisbon. The two sites differed on species composition and tree age. For each psyllid species, we estimated the population abundance and identified the associated fauna present in each survey date, covering the activity period of the psyllids. We also analyzed the sugar composition of honeydew and lerp produced by *G. brimblecombei*. With these results, we intended to further understand how sugar composition of these products may influence the associated fauna.

The three psyllid species differed on their seasonal activity. The two *Ctenarytaina* species were observed from January to June, whereas *G. brimblecombei* concentrated its activity between May and September. Several commensal and predatory native species were found associated with the psyllids, as well as two exotic Australian parasitoid species. The number of specimens and species richness of both native commensals and predators was significantly higher for *G. brimblecombei* in comparison with the two other psyllid species. The main sugar present on *G. brimblecombei* honeydew and lerps was fructose. This work offers contributions for the eucalypt plantation management towards the psyllid species, for the study of the how invasive honeydew producing species affect the native fauna and finally serving as a guideline for future studies in the honeydew sugar compositions and its effect on the fauna attractiveness of the honeydew.

Key words: eucalypts, psyllids, honeydew, biological invasion, native fauna.

Resumo

Nas últimas décadas tem-se registado um aumento significativo no número de introduções de espécies invasoras que se alimentam dos eucaliptos. Uma parte substancial destas espécies são insetos sugadores produtores de melada, em particular psilídeos (Homoptera: Psylloidea). A introdução destas espécies nas plantações de eucalipto tem, não só, um efeito negativo na produtividade das árvores, como impactos ecológicos resultantes da interação com a fauna nativa. Neste trabalho pretendemos estudar estas novas interações faunísticas com psilídeos produtores de meladas em plantações de eucaliptos em Portugal.

Estudámos três espécies invasoras de psilídeos, *Glycaspis brimblecombei*, *Ctenarytaina spatulata* e *Ctenarytaina eucalypti* durante um ano em dois locais na Tapada da Ajuda, Lisboa, com diferente idades das árvores. Para cada uma das espécies foi estudada a variação anual da atividade populacional e as interações com a fauna presente. Os insetos associados foram recolhidos, identificados e separados por três guildas: comensais, predadores e parasitóides. Analisámos a composição de açucares da melada e conchas do *G. brimblecombei,* com a intenção de melhor perceber como esta composição afecta a fauna associada às espécies.

As populações das três espécies foram diferentes na sua actividade sazonal, as duas espécies de *Cternarytaina* estiveram presentes no campo desde janeiro até junho, enquanto a *G. brimblecombei* concentrou a sua actividade entre maio a setembro. Várias espécies nativas comensais e predadoras foram encontradas associadas aos psílideos e também duas espécies exóticas australianas de parasitoides. O número de espécimes e riqueza de espécies das espécies nativas foram significativamente superiores para a *G. brimblecombei* comparativamente com as outras duas espécies. O principal açúcar presente na melada e nas conchas de *G. brimblecombei* foi a frutose.

Este trabalho contribui-o para o estudo dos efeitos despoletados na introdução de espécies produtoras de melada na fauna nativa nos eucaliptos, também para melhorar gestão das plantações de eucaliptos contras as espécies de psílideos e finalmente como guião para estudos futuros sobre a composição dos açucares das meladas e os efeitos na atracção da fauna feita pela melada.

Palavras-chave: eucaliptos, psilídeos, melada, invasão biológica, fauna nativa.

Resumo alargado

O género *Eucalyptus* é originário da Austrália e de ilhas próximas a norte, contendo mais de 800 espécies. Atualmente várias espécies de *Eucalyptus* são usadas mundialmente em plantações florestais. O eucalipto em Portugal é de grande importância, sendo as plantações florestais desta árvore as que mais cresceram nas últimas décadas, representando atualmente a principal ocupação da área florestal do país (ICNF, 2013).

As invasões biológicas são a segunda maior causa de ameaça de espécies nativas, depois da destruição de habitat, podem originar diferentes impactos, tanto de natureza ecológica, como na qualidade da vida humana. Em 2010, estimou-se que as pragas florestais e agrícolas invasoras custaram anualmente aos Estados Unidos da América cerca de 100 mil milhões de dólares, devido aos prejuízos causados e aos custos inerentes ao seu combate. Recentemente, tem-se verificado um aumento exponencial das introduções de espécies exóticas em todo o mundo, incluindo as plantações de eucaliptos, sendo o principal fator responsável por este aumento o comércio global. As trocas de mercadorias, em todo o mundo, oferecem às espécies invasoras uma nova forma eficaz de se dispersarem, aumentado exponencialmente as oportunidades de deslocação e estabelecimento em novas regiões.

A introdução de espécies exóticas não só representa um efeito negativo na produtividade das plantações de eucaliptos, que anteriormente tinham muito poucas pragas associadas, como conduzo ao estabelecimento de novas interações com a fauna nativa local. Várias espécies invasoras dos eucaliptos são insetos picadores-sugadores, da família Psyllidae (Hemiptera: Sternorryncha), que se alimentam de seiva e excretam melada. A melada é uma substância líquida e transparente, rica em açúcares, sendo usada como alimento, fonte de energia alternativa ou suplementar, por muitas espécies de insetos e aves, entre outras. Esta interação é do tipo comensalista, onde o inseto sugador não é influenciado pelo consumo da sua melada, ou tem benefícios de proteção, e o consumidor tem vantagem na obtenção de recursos. Adicionalmente, a presença destes insetos sugadores pode representar uma nova presa para predadores generalistas nativos, sendo neste caso uma interação de predação entre as espécies. Esta predação pode contribuir para a limitação natural das populações da espécie invasora.

Neste trabalho, estudámos as novas interações faunísticas resultantes da introdução de três espécies invasoras de psilídeos, recentemente introduzidas em Portugal, *i.e.*, *Glycaspis brimblecombei*, *Ctenarytaina spatulata* e *Ctenarytaina eucalypti*. Para tal, foi feito o acompanhamento sazonal da densidade populacional das três espécies de psilídeos, entre 30 de junho de 2015 e 30 de junho de 2016. Mensalmente, foi monitorizada a presença dos psilídeos nos eucaliptos amostrados. Durante o período ativo de cada espécie, estimou-se

semanalmente a proporção de folhas infestadas, em 10 ramos de cinco árvores selecionadas arbitrariamente, por local. O estudo foi feito em dois locais, com idades médias diferentes das árvores, um povoamento jovem e um maduro, localizados na Tapada da Ajuda, em Lisboa. Para cada espécie de psilídeo, recolheu-se, semanalmente e identificaram-se todos os elementos faunísticos associados a *G. brimblecombei.* Também foi feita a quantificação mensal dos seus ovos presentes nas folhas de 10 ramos. Foi também feita a análise da composição em açúcares da melada e conchas de *G. brimblecombei*. Estas análises foram realizadas atravéz da utilização de 12 diferentes soluções padrão de açúcares para conseguir verificar a presença dos compostos de açúcares nas amostras e quantificar a sua representação no total das amostras através do método HPLC-IR.

Verificou-se uma clara diferenciação das épocas de atividade das três espécies de psilídeos. As duas espécies de *Ctenarytaina* têm atividade entre janeiro e junho, enquanto *G. brimblecombei* desenvolve as suas populações entre Junho e final de Agosto. Estas diferenças de fenologia estão provavelmente associadas a diferentes respostas relativamente a fatores ambientais, como a temperatura, e/ou adaptação a diferentes fases fenológicas do hospedeiro.

Na comparação entre locais, verificou-se maior densidade dos psilídeos na parcela com árvores jovens. De igual modo, nas mesmas plantas, as folhas mais jovens são as mais atacadas. Sendo as folhas jovens as únicas folhas atacadas pelas duas espécies de *Cternarytaina* e preferidas pelo *G. brimblecombei*. Não se verificou preferência do ataque da *C. Spatulata* entre os dois eucaliptos estudados, *E. camaldulensis* e *E. rudis*. estando isto associado à próximidade filogenética das duas espécies vegetais, sendo ambas pertencentes à mesma secção Exertaria.

No total, foram identificadas 17 espécies na fauna associada aos psilídeos, incluindo 11 espécies comensais, quatro espécies de predadores e duas espécies de parasitoides. Estas 11 relações de comensalismo são todas derivadas do consumo da melada pelas espécies, nomeadamente de coleopteros, hymenopteros e dipteros. Os quatro predadores são o *Anthocoris nemoralis* (Hemiptera: Anthocoridae), *Chrysoperla carnea* (Neuroptera: Chrysopidae), *Coenosia attenuata* (Diptera: Muscidae), *Sphaerophoria scripta* (Diptera: Syrphidae). Finalmente os dois parasitoides encontrados foram o *Psyllaephagus bliteus* (Hymenoptera: Encyrtidae) da *G. brimblecombei* e o *Psyllaephagus pilosos* (Hymenoptera: Encyrtidae) da *C. eucalypti*. Registou-se maior abundância de espécimes comensais e predadores e riqueza destas espécies associadas a *G. brimblecombei,* comparativamente às associadas às espécies de *Cternayrtaina*. Estas diferenças podem ser atribuídas às diferenças sazonais na atividade dos psilídeos, assim como às diferenças na produção e

composição das meladas. Estimámos que a produção de melada por ramo infestado pelo *G. brimblecombei* é cinco vezes superior às das outras duas espécies estudadas, esta diferença será mais acentuada ao nível da árvore, visto que o *G. brimblecombei* infesta todas as folhas das árvores enquanto que as duas espécies de *Ctenarytaina* só infestam os ramos com rebentos foliares. Também se observou uma correlação positiva significativa entre o número de ovos de *G. brimblecombei* e a fauna associada.

Devido à quantidade limitada de melada recolhida foi apenas possível fazer a análise da composição em açúcares para *G. brimblecombei*. Verificou-se que as meladas e as conchas possuem algumas diferenças quantitativas nos açúcares identificados, sendo, todavia, a frutose e um outro açúcar não identificado, possivelmente isomaltose, os compostos dominantes. Foram verificadas diferenças quantitativas na composição de açúcares entre as meladas e as conchas, com uma maior abundância de frutose presente nas amostras de conchas e menor abundância do açúcar não identificado comparativamente com as amostras de meladas, uma maior quantidade de turanose nas conchas que na melada e finalmente a presença de ramnose nas meladas e ausência desta mesma nas conchas. As meladas de *G. brimblecombei* recolhidas em *E. camaldulensis* e *E. rudis* mostraram serem muito semelhantes, sugerindo não existirem diferenças relativas entre espécies de hospedeiros vegetais. Isto deve-se à próximidade filogenética das duas espécies vegetais, sendo as duas espécies pertencentes à secção taxonómica Exertaria, consequentemente a seiva do floema das duas espécies deverá ser semelhante e assim como o seu efeito na composição de açúcares das meladas ser semelhante.

Os nossos resultados oferecem um melhor compreendimento da dinâmica sazonal das três espécies de psílideos e dos potenciais agentes biológicos do controlo das populações dos psílideos, presentes nos eucaliptos. Futura monotorização e estudo dos impactos destes agentes nas populações dos psílideos conjuntamente com o conhecimento da dinâmica populacional das espécies de psílideos deverão ser importantes para futura gestão das plantações de eucalipto e controlo das populações de psílideos em Portugal. Este trabalho também contribuí-o para o estudo das interacções resultantes da introdução de espécies invasoras produtoras de melada com a fauna nativa nos eucaliptos, servindo como referência para trabalho futuro do estudo das relações comensalistas entre espécies invasoras produtoras de melada e a fauna de insectos nativos nos eucaliptos.

Finalmente, o nosso estudo na composição dos açúcares e quantificação das meladas servirá como guião para estudos futuros sobre a composição dos açucares das meladas e os efeitos na atracção da fauna feita pela melada.

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1. Introduction

The genus *Eucalyptus* consists of over 800 species originated from Australia and some islands to the north of it. At present, eucalypts are one the most used trees worldwide in forest plantations for the production of timber, pulp, fuel wood, pharmaceutical compounds, fragrance and flavor use, ornamental purposes, soil conservation and in honey production (Coppen, 2002).

The spread of *Eucalyptus* species all around the world occurred after the Cook expedition in 1770, in which eucalyptus trees were collected by the botanist Sir Joseph Banks, and brought to London for study. Later, in the 19th century they were spread all over the world. In the U.S.A., eucalypts were being planted for the first time in Southern California at around 1850 (Paine at al. 2010).

Eucalypt species cover a wide climatic and edaphic range of conditions in their natural habitat, which allows them to be planted in a very wide range of distant and varied locations world-wide. The highly diverse pool of genes of the *Eucalyptus* genus is another reason behind their successful introduction in so many varied locations all over the globe. Finally, their fast-growing nature makes them one of the best tree species for biomass growth in a short period of time, and also offers the possibility of a much quicker economic return from their use, comparatively to most of the other forest tree species (Coppen, 2002).

According to Iglesias-Trabado and Wilstenmann (2008), 19,609,670 ha of the world surface are planted with eucalypts. The three biggest contributing countries are India with 3,942,600 ha, Brazil with 3,751,857 ha and China with 2,609,700 ha. Portugal and Spain have very similar eucalypt plantation areas of 647,000 ha and 640,000, respectively. The main species used in Portugal is *Eucalyptus globulus* Labill, which was first introduced in Portugal in 1854 (Alves *et al*., 2007). According to ICNF (2013), the area of eucalypts in Portugal increased by 13%, between 1995 and 2010. In 2010, eucalypt forest reached 812,000 ha, representing 26% of forest area in the continental territory.

For a long period, *Eucalyptus* were introduced in the new areas without their herbivores. The first *Eucalyptus* feeding invasive species found outside its native range was *Phoracantha semipunctata* Fabricius, reported in New Zealand in 1870 (Withers, 2001). Later, this species spread throughout the world. Until 1970, only 16 pest species were reported to be found in *Eucalyptus* plantations outside their native range (Paine et al., 2010; Hurley *et al*., 2016). Of those species, only seven were reported outside of the oceanic continent during that period.

Since 1970, numerous new invasive *Eucalyptus* feeding species were reported world-wide on eucalypt plantations outside of Australia. A higher rate of these invasive species introductions

occurred recently, especially after the 80's (Paine et al., 2010; Hurley *et al*., 2016; Manshfield, 2016). In total, 28 new invasive species were reported world-wide since 1970, which actually represent 44 invasive species reported outside their native range (Hurley *et al*., 2016). These species are distributed among 16 different families from the orders Hemiptera, Coleoptera, Hymenoptera and Lepidoptera and include five different feeding guilds: sap suckers, defoliators, gall makers, wood borers and leaf miners (Paine et al. 2010; Hurley *et al*., 2016).

Biological invasions are the process in which an alien species invades a new region and manages to establish, having been facilitated by human activity or natural ways (Zhang and Chen, 2011). When these alien species manage to establish in a new area and have negative impact to the local environment, these are considered invasive species. Invasive alien species can originate different types of negative impacts upon the human living, such as economic, environmental and social impacts (Burgiel *et al.*, 2006).

Biological invasions are considered as one of the three most difficult environmental problems in the world, being ranked second, right behind habitat degradation as a source of species endangerment (Wilcove et al., 1998). They are estimated to have cost the U.S. economy over 100 billion dollars in losses per year, considering both damage caused and control costs (Pimentel *et al*., 2000).

One of the most important factors of the recent increase in worldwide biological invasions is global trade. Being responsible for the prosperity and many benefits to societies all around the world, it also created a new and very efficient pathway for alien organisms to reach and colonize new areas (Burgiel *et al.*, 2006). The transfer of these alien organisms is often unintentional, but many times it has been done intentionally (Burgiel *et al*., 2006). Another factor is the recent increase of the traveling of people around the world, which similarly to global trade also creates a pathway for alien organisms to be introduced to new areas, either intentionally or not. Zhang and Chen (2011) verified that the number of invasive species present in a country are positively correlated to its level of economic development and trade activity.

The recent and future increase of introductions will lead to an increase to the probability of the invasive alien species becoming established in the new environments. But the successful establishment of these alien species in the new areas is dependent on many different factors, including the climatic and environmental conditions, and the species characteristics (Zhang and Chen, 2011).

Invasive insect pests can have several ecological impacts (Kenis *et al*., 2008), including the effects caused by the interactions of the invasive species with different native insect species,

which can affect the biodiversity of the ecosystems through several different direct or indirect mechanisms explained in detail by Kenis *et al*. (2008).

The ecological effects originated by insect pests can be minor, affecting only a few elements of the ecosystems, usually through simple trophic interactions. However, they may also cause major ecological impacts. Normally, a cascading effect of ecological consequences triggered by the pest species can affect not only the biological elements of the ecosystem, but also the ecosystem processes (Kenis *et al*., 2008).

Biological invasions can also have a positive contribution for the native biodiversity, through the creation of new interactions between the native fauna and the invasive species. These new interactions can be beneficial for the native fauna, such as commensal and predatory interactions with the invading species. These commensal interactions have been reported in the past to occur with honeydew producers, benefitting honeydew feeders in native fauna, including not only insects but also other animals, such as birds (Douglas, 2006). The introduction of a new element to the fauna can also represent the addition of another prey to the diet of the local native generalist predators.

Sap suckers (Insecta, Hemiptera) represent a large proportion (41%) of the eucalypt-feeding invasive species. The first sap-sucker species reported outside its native range was *Ctenarytaina eucalypti* Maskell. It is a psyllid (Sternorryncha, Psylloidea) whose presence was reported in New Zealand, in 1889 (Withers, 2001). Presently, 18 sap-sucking invasive species are reported outside their native ranges, including 14 psyllids. *C. eucalypti* was also the first psyllid species reported in Portugal, in 1971 (Figo and Silva, 1977).

Sap suckers may feed either on the phloem or xylem of the host plant. Sap-suckers of the sub-order Sternorryncha, mostly aphids (Aphidoidea), scale insects (Coccoidea), and psyllids, are distinguished from the other hemipteran by the rearward position of the mouthparts adapted to feed on phloem sap (Sharma *et al*., 2013). Most of these insects excrete honeydew.

Unlike the other two types of sugar-rich foods that plants provide to animals (nectar and fleshy fruits), the phloem sap did not evolve in a way to be accessible to animals as food. The relationship between sap sucker and host plants is an antagonistic one. Although phloem sap is an undefended, nutrient-rich food source, accessible to sap suckers, it is not meant to be consumed, from the plant point of view (Douglas, 2006).

One of the problems of the phloem sap as food is the low nitrogen quality related to the low ratio of essential amino acids to non-essential amino acids, 4 to 20 times lower than in animal proteins. This barrier is overcome by sap-feeders through symbiotic micro-organisms which help complementing the diet of the host (Douglas, 2006).

The second obstacle of using phloem sap as food is the very high concentration of sugars, resulting in a very high osmotic pressure, up to five times higher than that in the insect´s body fluid (Douglas, 2006). For this reason, sap-feeding insects possess sucrose-transglucosidase activity in their gut, transforming excessive ingested sugars into long-chain oligosaccharides, which are excreted as honeydew. The transformation of the ingested sucrose into oligosaccharides will reduce the osmotic pressure of the gut.

Honeydew is a clear looking liquid, with syrup like properties and a very high sugar content which is expelled during the feeding process of the sap-feeding insect, making it an easy source of energy for other animals (Douglas, 2006). This food source attracts not only commensal relationships between honeydew producing sap-feeders and honeydew feeding fauna, but also mutualistic relationships, which are very common and well-studied, such as those between honeydew producing insects and ants. In this relationship, also called trophobiosis, the ants consume the honeydew and in return protect the honeydew producers, from predators or parasitoids, clean fungi growth in the colony and remove infected or dead members from the colony (Buckley, 1987). Oddly enough, this relationship with the ants in trees can, sometimes, end up turning the previously said antagonistic relationship between the host trees and the sap feeding insects into a three-way mutualistic relationship, where the sap-feeder feeds off the tree, the ants consume the honeydew produced and in return tend the sap-feeding insects and at the same time protecting the tree from herbivores (Douglas, 2006). Finally, honeydew may also attract predators and parasitoids of honeydew-producing insects. These natural enemies can use the honeydew, as an alternative food source (Wäckers et al, 2008), but also to locate their prey (Bouchards and Cloutier, 1984; Meiracker *et al*., 1990).

Psyllids are small sized sap-sucking insects, usually monophagous or oligophagous (Hodkinson, 1974). There are about 380 described species of psyllids in Australia (Holis, 2004). The subfamily Spondyliaspinidae is almost exclusively restricted to Australian native species which use Myrtaceae as host plants, including *Eucalyptus* (Santana and Burckhardt, 2007).

Injury originated by psyllids feeding on eucalypt trees include the development sooty mould and phytopathogenic fungi on the leaves and buds, in association with honeydew excreted by psyllid nymphs, as well as shoot dieback, loss of apical dominance, decrease of tree growth and super sprouting of lateral buds originated from nymphal and adult feeding (Santana *et al*., 2005).

The number of generations psyllids are able to complete per year depends on climatic conditions. With favorable climate they tend to be multivoltine species, having several overlapping generations each year (Burckhardt, 1994; Hollis, 2004).

Ctenarytaina eucalypti is native to southeast Australia and affects mostly *E. globulus* (Figo and Silva, 1977). Effectively, this species has a small range of host eucalypt species, all belonging to the section Maidenaria, which were described by Santana *et al*. (1999).

Ctenarytaina spatulata Taylor originates from southeast Australia and was first reported outside of its origin in California, in 1991 (Gill, 1998). Afterwards, it has been reported from many other countries in Europe and South America, but also from New Zealand (Taylor, 1997; Withers, 2001; Valente *et al*., 2004). This species has a life cycle of approximately 45 days from egg to adult, at temperatures around 20ºC (Santana and Zanol, 2006). Unlike *C. eucalypti*, it is associated with different *Eucalyptus* species. Thirteen species of *Eucalyptus* were reported by Santana (2003) as hosts of *C. spatulata* but many more have been mentioned in past literature.

Glycaspis brimblecombei Moore also originates from Australia and was first reported outside its native range in California, in 1998 (Brennan *et al*., 1999). It has spread to many others countries, in North and South America, Africa and Europe (Firmino *et al*., 2009; Valente and Hodkinson, 2009; Hurley *et al*., 2016). Unlike the other two psyllid species mentioned, the nymphs live underneath a waxy structure called lerp. The lerp is constructed by the nymphs for their protection (Sharma *et al*., 2013). Their life cycle, from egg to adult, is between 65 to 70 days (Sharma *et al*., 2013). According to Firmino (2004), 26ºC is the optimal temperature. *Glycaspis brimblecombei* has a very wide range of host eucalypt species. Still, *E. camaldulensis* and *E. tereticornis* are considered the preferred host species (Brennan *et al*., 1999; Hollis, 2004). Damage originated by *G. brimblecombei* on eucalypts is similar to that of the other two previously mentioned psyllid species. Nevertheless, this species seems to be much more aggressive to the host plants. High populations can lead to tree mortality, due to premature defoliation (Queiroz *et al*., 2012). It is considered the most damaging psyllid species introduced in California (Brennan *et al*., 1999).

Regarding new interactions between native fauna and the introduced invasive eucalypt psyllid species, as far as we know, despite few observations, there is no systematic and thorough study. Some generalist native predator species preying on the invasive psyllid species have been reported in a few works (Brennan *et al*., 1999; Michaud, 2002, Valente and Hodkinson, 2009; Laudonia et *al*., 2014). But none of these works provide systematic data. Furthermore, no work has been done yet for the commensal interactions between native species and the invasive psyllids. The study of sugar composition of psyllid honeydew and lerps has not attracted much attention so far. The only contribution we found in the literature was that of Basden (1970), where the composition of *Eucalyptolyma maidenii* Froggatt lerps was

compared to that of plant starch. In the same work, sugar components of the honeydew excreted by *E. maidenii* and *Eriococcus coriaceus* Mask were also compared.

With this work we aimed at studying the seasonal dynamics along the year of the three invasive eucalypt-feeding psyllids, *G. brimblecombei, C. spatula* and *C. eucalypti* to better understand their population's dynamics and their possible interactions with native fauna. With the identification and quantification of elements of the native fauna according to guilds we further aimed at establishing the importance of different trophic relationships. Finally, we also intended to study the sugar composition of honeydew and the lerps produced by the psyllid species, which might be a key-factor for some of the native fauna interactions.

2. Materials and Methods

2.1 Study sites

The study was conducted in two sites, at the Instituto Superior de Agronomia (ISA), Tapada da Ajuda, Lisbon. The first site is an arboretum composed of 30 species of *Eucalyptus*, from now on called “Arboreta” (38°42'55.1"N 9°11'32.5"W). The Arboreta occupies approximately 1 ha and was planted in 2007, with the purpose to provide food for the koalas of the Lisbon Zoo. Some plants died in the first year and were then replaced. The trees on this site were thus about 7-9 years old. The second site is called “Eira Velha” (38°43'05.7"N 9°11'16.1"W), and is composed mainly by eucalypts from Exertaria section (e.g. *E. camaldulensis*, *E. diversicolor*, *E. tereticornis*) and occupies approximately 2 ha, with eucalypts that are more than 30 years-old. The climate in Lisbon is Mediterranean, according with the climatic classification of Kõppen, Csa, characterized by cold and rainy winters and hot and dry summers.

2.2 Seasonal activity of the three psyllids

We surveyed the activity of the three studied psyllid species during one year, from 30th of June 2015 until 30th of June 2016. We registered the presence of the psyllid species at a regular monthly interval in the Arboreta and Eira Velha sites, inspecting five randomly selected trees per site. Whenever psyllids were present in a tree, we monitored their activity in a weekly basis until their disappearance, by estimating the proportion of leaves infested with psyllids.

Since the psyllid species differ on their host plants and type of leaf infestation, and were also present in different periods, each species was surveyed according to a specific protocol, detailed below. The leaf inspection approaches were all non-destructive

2.2.1 *Glycaspis brimblecombei*

This psyllid was only surveyed on *E. camaldulensis* trees. For the weekly monitoring, in 2015, 10 branches of 40-50 cm long were inspected from five randomly selected trees, during the morning, two branches from opposite sides of each tree, on each date and site. Additionally, in the afternoon of the same date, 10 similar branches were inspected from other five randomly selected trees, corresponding to total of 20 branches per survey date, except for the first date, in which the sampling was only carried out in the afternoon.

In 2015, during the final months of the species activity, the observation samplings were done at the end of each month and only once for each survey date. In 2016, the species activity sampling was done every two weeks, only once in each survey date.

2.2.2 *Ctenarytaina spatulata*

Weekly samplings were not always possible, due to some heavy raining weeks, and so samplings were postponed to later dates or anticipated to earlier dates. This anticipation was possible with the consulting of the Instituto Português do Mar e Água (IPMA) Website for the weekly weather forecast.

This psyllid was surveyed on *E. camaldulensis* and *E. rudis* trees, in the Arboreta and only on *E. camaldulensis* in Eira velha. In each date and site, 10 branches from five randomly selected trees with new flushing leaves were inspected per eucalypt species by observing two branches from opposite sides of each tree. Unlike with *G. brimblecombei*, the sampled branches needed to have young shoots. The number of leaves infested and not infested with psyllids was counted.

2.2.3 *Ctenarytaina eucalypti*

This species was only surveyed in the Arboreta, on *E. globulus*. As described for the other species, in each date, we inspected 10 young branches with shoots and young leaves from five randomly selected trees, by observing two branches from opposite sides of each. Like with *C. spatulata* the sampled branches needed to have young shoots from the juvenile leaves of *E. globulus*. The number of leaves infested and not infested with psyllids was counted.

2.3 Leaf position

During the survey of *G. brimblecombei,* from June to August of 2015, monthly samplings were carried out in the two sites in order to count the number of eggs of the psyllid on leaves of *E. camaldulensis*. In each site and date, 10 branches were inspected from five randomly selected trees, by observing two branches in opposite sides of each tree. Egg counting was done in the field, with the help of a head-wearing magnifying glass (1.8X to 4.8X magnification) and a hand counter.

Each branch was divided in three sections, to separate the leaves according to their age, as following: i) apical leaves, corresponding to the first third of the branch; ii) median leaves, corresponding to second third; and iii) distal leaves, corresponding to the basal third. This leaf inspection approach was also non-destructive.

2.4 Associated fauna

The associated fauna was surveyed on the same dates and simultaneously with the weekly samplings of the psyllids seasonal activity survey. For *G. brimblecombei*, the samplings were done twice for each date, due to the temperature variations through the day. For the two

Ctenarytaina species, fauna sampling was conducted only once for each date, during the warmer period of the day, between 10:00 and 13:00, due to the much lower daily temperature variation, in their survey period.

To survey the associated fauna we observed for two minutes each selected branch. All insects present on the leaves at the time of the observation, which were confirmed to be truly associated with the psyllid species, either by their feeding activity or behaviour, were collected, using an entomological vacuum device or manually, from the leaves. For rapid flying insects, we used a flask to catch them in mid-air. If any insect managed to escape, its presence was recorded anyway. Eggs of Neuroptera and Syrphidae, which were easily seen and identified on the leaves, were also counted. The collected insects were introduced within a flask (one per branch). In the laboratory, the insects were killed in the freezer and then preserved in ethanol 70%. Taxonomic identifications were carried out under a stereomicroscope SZM-LED1, of (7x - 90X magnification). The following keys were used: Goulet and Huber (1993), and Chinery (1997), for Hymenoptera; Scudder and Cannings (2006), for Diptera, and Stubbs and Falk (1983), in the case of Syrphidae; Choate (1999), for Coleoptera; Choate (2010), for Hemiptera. Additionally some insects were identified with the aid of André Garcia and Vera Zina.

All identified specimens were classified according to their type of relationship with psyllids in three functional groups or guilds: commensals, predators and parasitoids.

2.5 Honeydew

2.5.1 Collection

2.5.1.1 *Glycaspis brimblecombei*

For the *G. brimblecombei* solid honeydew collection, we manually collected lerps from infested *E. camaldulensis* trees located at Tapada da Ajuda Campus, between the buildings Azevedo Gomes and Ferreira Lapa (Lerps A), as well as from infested leaves of *E. camaldulensis* maintained in cages in the laboratory (Lerps B). The collected lerps were conserved in the freezer (-18ºC).

For the liquid honeydew, we collected branches of eucalyptus infested with *G. brimblecombei*, from both *E. camaldulensis* and *E. tereticornis*, in the Arboreta. In the laboratory the honeydew overflowing from the lerps or beneath the lerps was collected, using disposable capillary micropipettes (Hirshmann, mini caps of 5 µL, 9000205). The collected honeydew was conserved in the freezer (-18ºC).

After the liquid honeydew collection, the leaves with healthy and productive nymphs were kept in dry bags, in the refrigerator, in order to stop the crystallization process of the honeydew and reduce the metabolism of the nymphs, but allowing them to survive, while still producing honeydew during the night. The next morning, we collected again the liquid honeydew that some nymphs had produced. This procedure was repeated until *G. brimblecombei* population fall, in Setember. Both the solid and liquid honeydew samples were conserved in the freezer for later analysis.

We estimated the amount of honeydew per leaf and extrapolated it for the all branch, based on the average number of infested leaves per branch.

2.5.1.2 *Ctenarytaina spatulata* and *Ctenarytaina eucalypti*

For *C. eucalypti* honeydew, we collected infested branches of *E. globulus*, as for *C. spatulata* we used infested branches of *E. camaldulensis* and *E. rudis*.

The infested branches of eucalypts were transported to the laboratory in dry bags, where the honeydew was collected with the help of laboratory needles. This different procedure was used due to the less fluid nature of the honeydew produced by *Ctenarytaina* species.

The collected honeydew was conserved in the freezer, grouped per host-plant species. We then estimated the amount of honeydew as described for *G. brimblecombei*.

The branches with better leaf shoots were kept in water cups in laboratory conditions. Beneath these cups we used a plastic base (40cm x 40 cm) to capture all the falling honeydew, expelled by the nymphs.

2.5.2 Sugar composition analyses

The collected amounts of honeydew from *C. spatulata* and *C. eucalypti*, were not enough to perform the analysis. Therefore, only the sugar composition of *G. brimblecombei* honeydew and lerps was studied.

Because of the different quantities of the samples, two repetitions were done for the two lerp samples and for the samples of *E. tereticornis*, whereas eight samples were considered for *E. camaldulensis*. The final results are presented as mean values.

Standards and chemicals

Pure fructose, glucose, sucrose, melezitose, turanose, maltose, xilose, rhamnose, arabinose, melebiose, trehalose and erlose (Table 1) were used to prepare standard solutions to identify and quantify the individual sugar components in the samples, by HPLC-IR (Bogdanov et al. 2002).

Milli-Q water (Millipore) was used in all preparations.

Table 1 - Chemical standards used (CAS number, supplier and purity).

Chemical	CAS number	Supplier	Purity (%)
Fructose	57-48-7	Sigma	≥99
Glucose	50-99-7	Sigma	≥99.5
Sucrose	57-50-1	Sigma	≥99.5
Melezitose	207511-10-2	Sigma	≥99
Turanose	547-25-1	Sigma	≥98
Maltose	6363-53-7	TCI	≥98
Xilose	58-86-6	Alfa Aesar	≥98
Ramnose monohydrate	10030-85-0	Alfa Aesar	≥99
Arabinose	10323-20-3	Sigma	≥98
Melebiose	585-99-9	Sigma	≥99
Trehalose	6138-23-4	Sigma	≥98.5
Erlose	13101-54-7	Sigma	≥97
Rafinose pentahydrate	17629-30-0	Flika	≥99
Acetonitrile, super gradient (water <30 ppm)	75-05-8	VWR	99.9
Methanol HPLC grade	67-56-11	Fisher	99.99

After solution filtration, the sugar content was determined by High Pressure Liquid Chromatography (HPLC) with RI-detection. Peaks were identified on the basis of their retention times. The quantification was performed according to the calibration curve method.

Sample preparation for analysis

Given the low quantity of sample it was not possible to weigh a quantity to prepare the solution to analyse. In this case the samples in the Eppendorf were diluted with a solution of water and methanol in a ratio of 3:1.

The Total Soluble Solids (TSS) in ºBrix (°Bx) were evaluated in the final solution. The Brix degrees measure the dissolved solids concentration in a solution and were expressed as percentage (w/w). The TSS or sugar content measures included carbohydrates, organic acids, proteins, fats and minerals. Membrane filters (HPLC certified) for aqueous solutions with pore size of 0.45 mm were used before injection in the HPLC.

High Performance Liquid Chromatography (HPLC)

The High Performance Liquid Chromatography consisted of a pump, a sample applicator, temperature regulated RI-detector thermostated at 30ºC, and temperature regulated column oven at 30ºC and an integrator.

Analytical stainless-steel column, purospher® STAR – NH_2 with 4 mm in diameter, 250 mm length and with 5 mm particle size, were used (adapted from Bogdanov et al. 2002).

The following conditions were used to give satisfactory separation of the different standards: flow rate: 1.3 ml/min; mobile phase: acetonitrile: water (80:20, v/v); column and detector temperature: 30ºC; sample volume: 10 ml.

Before use, some experiments were carried out to ensure that all sugars analysed could be separated.

Calculation and expression of results

The honeydew sugars were identified and quantified by comparison of the retention times and the peak area of the honey sugars with those of each calibration curve of standard sugars. The mass percentage of the sugars, W, to be determined of fructose, glucose, in g/100 g of TSS. Table 2 summarizes the retention time of each sugar as well as the detection limit (LD) and quantification limit (LQ) measured in g/100 mL of solution and g/100 g of TSS.

Table 2 – Values of retention time, LD and LQ for each sugar quantified in the honeydew.

Sugar	**Retention time (min)**	**LD**	**LQ**	**LD**	**LQ**
		(g/100 mL)		(g/100 g of TSS)	
Rhamnose	3.83	0.003	0.010	0.057	0.170
Xylose	4.24	0.013	0.043	0.261	0.870
Arabinose	4.96	0.002	0.006	0.039	0.129
Fructose	5.46	0.012	0.039	0.232	0.773
Glucose	6.42	0.024	0.081	0.484	1.612
Sucrose	9.45	0.005	0.016	0.099	0.329
Turanose	10.45	0.001	0.003	0.017	0.057
Maltose	11.55	0.010	0.033	0.199	0.663
Melibiose	12.69	0.002	0.006	0.034	0.112
Trehalose	15.27	0.003	0.008	0.050	0.168
Melezitose	18.32	0.001	0.004	0.025	0.083
Erlose	22.06	0.002	0.008	0.049	0.165

2.6 Statistical analysis

2.6.1 Infestation data

To compare the proportion of infested leaves by *G. brimblecombei* and *C. spatulata* between the two study sites, we used a generalised linear model with binomial distribution and logit link function, considering the factors site and time. For both species, we only considered *E. camaldulensis* trees. The probability of leaf infestation, denoted as p, was estimated by maximum likelihood. To test the hypothesis that the two study sites have the same probability of leaf infestation, $p_{Arboreta} = p_{Eira\text{-}Velha}$, we used a 5% significance level.

To compare the proportion of *C. spatulata* infested leaves, between two eucalypt trees species, *E. camaldulensis* and *E. rudis*, both from the Arboreta study site we used a generalized linear model as described above. The probability of leaf infestation, denoted as p, was estimated by maximum likelihood. We tested the hypothesis that the two host species have the same proportion of leaf infestation, $p_{camaldulensis} = p_{rudis}$, with a 5% significance level.

2.6.2 Egg data

We tested if the leaf age, using its positioning in the branch (apical, median or basal), was a significant factor influencing the distribution of *G. brimblecombei* eggs among *E. camaldulensis* leaves. We estimated the mean number of eggs, using a negative binomial distribution and log link function. The mean number of eggs, denoted as μ, was estimated by maximum likelihood. We tested the hypothesis that the three different leaf positions have the same mean number of eggs, $\mu_{apical} = \mu_{median} = \mu_{basal}$, with a 5% significance level.

2.6.3 Fauna data analyses

We compared the mean number of commensal and predator specimens collected per survey sampling between the three psyllids using a t-test for difference in means. The richness of commensal and predator species between the tree psyllid species was compared using a Chi-square test.

Using the monthly mean abundance of specimens for each different functional group we studied the correlation between the abundance of the fauna functional groups and the mean number of eggs of *G. brimblecombei* in eucalypt leaves for the three months studied (June, July and August) (N=6). The significance of the spearman correlation coefficient was checked using a one-tailed t-test with a 5% significance level.

2.6.3 Honeydew data analyses

For each sugar we compared its quantified concentration levels in the four samples using a univariate ANOVA analysis.

All of the statistical analyses was done using the IBM SPSS 21 programme. Standard errors are abbreviated as S.E. and degrees of freedom as df.

3. Results

3.1 Seasonal activity of three psyllid species

Glycaspis brimblecombei was already present in the two study sites at the start of the seasonal activity survey in the 30th of June, where the weekly study surveys were done until the 24th of August when the species activity was very low. From this date on, the species was only monitored at the end of each month, having disappeared in the 21st of November of 2015. In 2016, the species activity was first detected in the 23rd of May and its activity was monitored in two more survey dates, in the 14th and 30th of June, when the year survey ended. The *C. spatulata* species activity was first detected in the two study sites in the 21st of January of 2016, while the *C. eucalypti* species activity first detection occurred later in the Arboreta in the 28th of January. These two species were monitored until the end of their activity, in the 30th of June.

Two periods of psyllid activity can be distinguished along the year (Figure 1). The presence of *G. brimblecombei* was observed from May to October with a peak in the end of June, whereas the two *Ctenarytaina* species were observed between February and June, without a clear population peak. *Glycaspis brimblecombei* population fell significantly in August and finally almost completely disappeared in October and November (Figure 1). The two psyllid genera only co-existed during a small period of time, from the end of May till the end of June.

The meteorological conditions during the analyzed period were characterized by a mild weather with an overall mean temperature of 17.9 ºC. The hottest months, with a daily mean temperature over 20 ºC, were June, July, August and September of 2015 and June in 2016. The coldest months with a daily mean temperature below 15 ºC, were December in 2015 and January, February and March in 2016.

Rainfall was concentrated in October in 2015 and from January to May in 2016 (Figure 2).

The end of *G. brimblecombei* activity coincided with the appearance of rainfall after a very long period with almost no rain, and the decrease in temperature (Fig 2). The activity of the two *Ctenarytaina* species started in January, when the daily mean temperature was 13.5 ºC and the minimum daily temperature was 10.6 ºC. The two months with highest activity of *Ctenarytaina* species (January and February) coincided also with monthly rainfall above 100 mm (Figure 2). In fact, rainfall was present during the whole period of their activity with a decrease in March and again higher values in April and May.

In May, the populations of the two *Ctenarytaina* species started falling and ended in June when *G. brimblecombei* population was just starting to rise. During this period, there was a significant rise of the temperature, with a mean of 22.2 ºC (Figure 2).

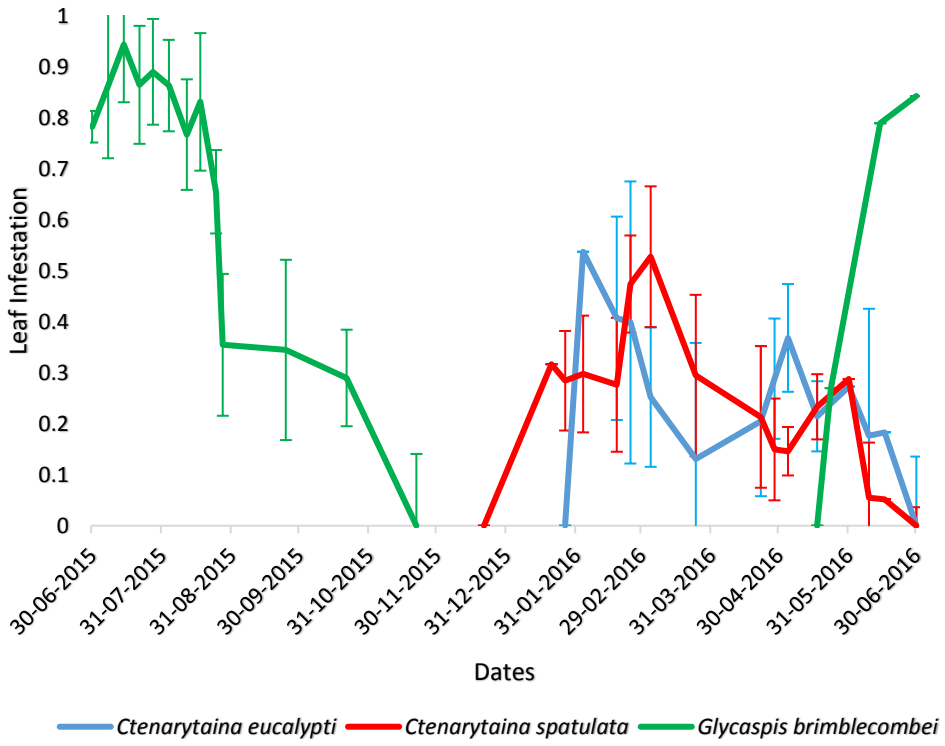

Figure 1 – Proportion (mean ±1 SE) of leaf infestation of three psyllid species, *Glyscaspis brimblecombei*, *Ctenarytaina spatulata* and *Ctenarytaina eucalypti*, during a one year survey in the Arboreta, Tapada da Ajuda, Lisbon.

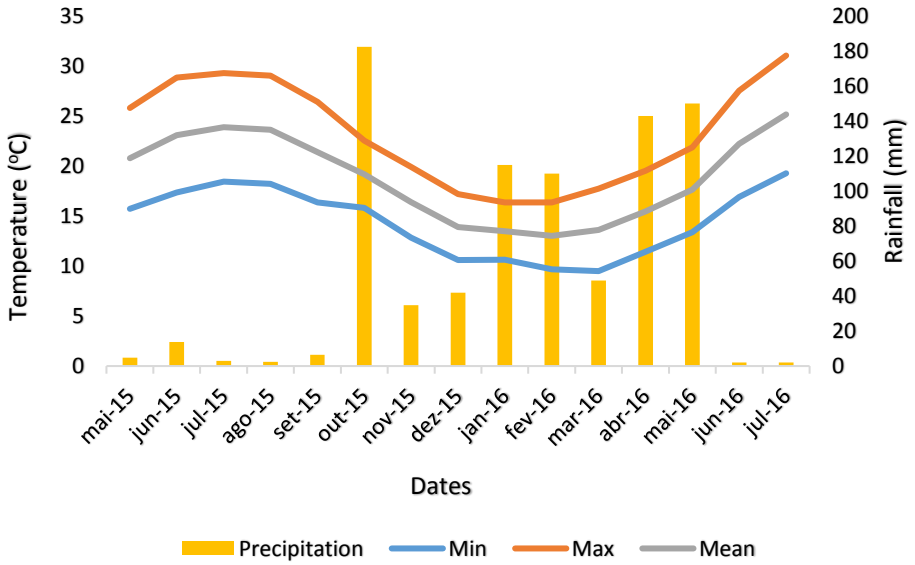

Figure 2 – Daily mean, maximum and minimum temperatures and precipitation during the studied period, from June of 2015 till July of 2016, in Lisbon, Portugal. Values obtained from IPMA.

3.2 Differences between sites

The proportion of infested leaves by *G. brimblecombei* in *E. camaldulensis* was significantly different between the two study sites *(*p-value=0.001; df=1; Chi^2=20.83). Overall, infestation was higher in the Arboreta than in Eira Velha (Figure 3). The proportion of infested leaves was not constant over time (p-value=0.001; df=8; Chi^2=27.37).

Figure 3 – Proportion (mean ±1 SE) of leaf infestation by *Glycaspis brimblecombei* in *Eucalyptus camaldulensis* trees in the Arboreta and in Eira velha between 30/06/2015 and 24/08/2015 (N=20).

The proportion of infested leaves by *C. spatulata* in *E. camaldulensis* was also significantly different between the two study sites *(*p-value=0.001; df=1; Chi^2=137.95), with much higher leaf infestation values in the Arboreta in comparison with Eira Velha. Leaf infestation was not constant over time (p-value=0.001; df=14). In both study sites the species activity ended in June, but in Eira Velha clear reduction in infestation level was observed after March, which did not happen so markedly in the Arboreta (Figure 4).

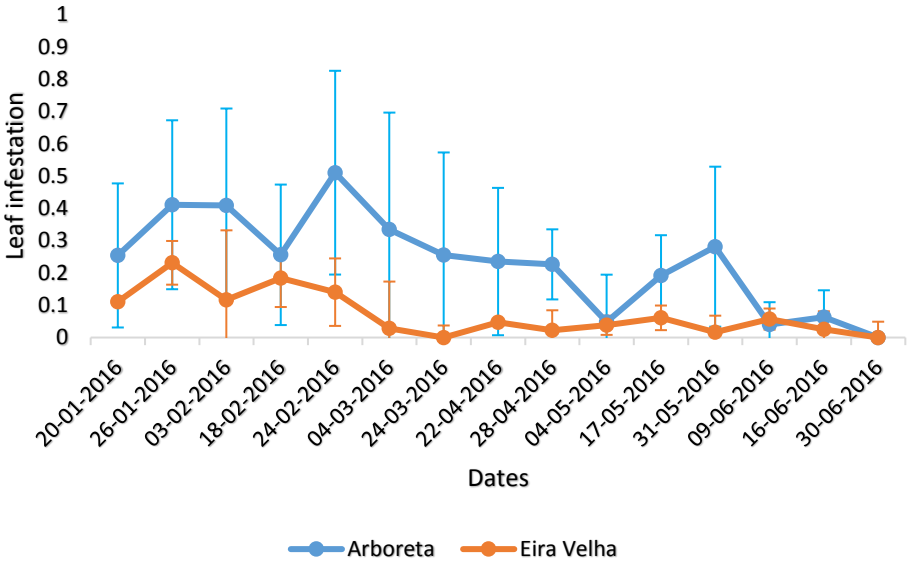

Figure 4 – Proportion (mean ±1 SE) of leaf infestation by *Ctenarytaina spatulata* in *Eucalyptus camaldulensis* trees in the Arboreta and Eira velha from 20/01/2016 to 30/06/2016 (N=10).

3.3 Differences between host species

Leaf infestation of *C. spatulata* was not significantly different between the two eucalypt host species, *E. camaldulensis* and *E. rudis* (p-value=0.297).

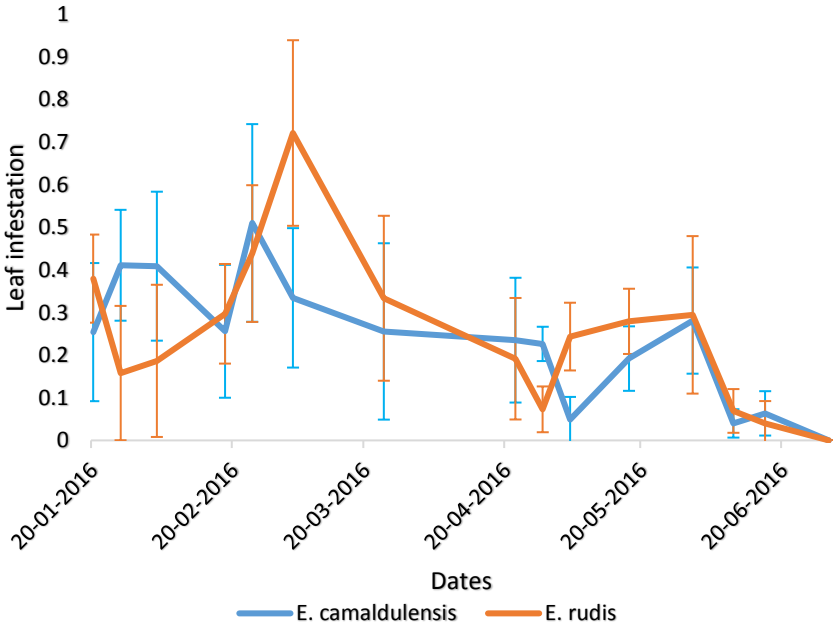

Figure 5 – Proportion (mean ±1 SE) of leaf infestation by *Ctenarytaina spatulata* in *Eucalyptus camaldulensis* and *Eucalyptus rudis* in the Arboreta from 20/01/2016 to 30/06/2016 (N=10).

3.4 Leaf position

For *G. brimblecombei,* the mean number of eggs on the apical leaves (mean=4.58 ± 0.64 SE) were not significantly different from that on median leaves (mean=5.07 ± 0.71 SE) (p-value=0.268; df=1; Chi^2=1.228). On the contrary, the older leaves, *i.e.* those located on the basal part of the branch, had significantly lower number of eggs (mean=2.54 ± 0.46 SE), in comparison with apical and median leaves (p-value=0.001; df=1; Chi^2=23.19).

3.5 Associated Fauna

3.5.1 *Glycaspis brimblecombei*

During survey 101 different insect specimens from 5 different insect orders, 13 families and 15 species were found associated with *G. brimblecombei* (Table 3).

Table 3 – List of the species of insect fauna found associated with *Glycaspis brimblecombei* during the weekly survey, ordered by their functional group, taxonomic position and origin.

Guild	Order	Family	Species	Origin	N
Commensals	Coleoptera	Mordellidae	Unidentified	Native	3
		Scraptiidae	Unidentified	Native	1
		Melyridae	Unidentified	Native	1
		Curculionidae	Unidentified	Native	1
	Diptera	Muscidae	Musca sp.	Native	5
	Hymenoptera	Apidae	*Apis mellifera* L.	Native	2
		Formicidae	Plagiolepis *schmitzii Forel*	Native	29
			Crematogaster scutellaris Oliv.	Native	1
		Ichneumonidae	Unidentified	Native	3
		Vespidae	*Polistes gallicus* L.	Native	4
			Vespula germanica Fabr.	Native	1
Predators	Neuroptera	Chrysopidae	*Chrysoperla carnea* Steph.	Native	33
	Hemiptera	Anthocoridae	*Anthocoris nemoralis* Fabr.	Alien	5
	Diptera	Muscidae	*Coenosia attenuata* Stein	Native	1
Parasitoid	Hymenoptera	Encyrtidae	*Psyllaephagus bliteus* Riek	Alien	16

The functional group of commensals was the most frequent, with a total of 51 specimens, distributed among 11 different species, from nine different insect families within the orders Coleoptera, Diptera and Hymenoptera.

The functional group of predators was represented by a total of 39 specimens. Still, all these specimens belonged only to three different species, a chrysopid, an anthocorid and a predator fly.

Finally, the functional group of parasitoids was represented by a single insect species, with 16 specimens. This was *P. bliteus*, a parasitoid of *G. brimblecombei,* native to Australia which was recently introduced in Europe.

The mean number of specimens collected was highest in 30th June 2015 (Figure 6), which corresponded to the peak of *G. brimblecombei* activity, and decreased thereafter, being lowest in August, when the *G. brimblecombei* population was falling. The three functional groups had a similar trend in time, with the parasitoids falling earlier than the other functional groups, not having been found in August, which should be related with the fall of the reproduction of *G. brimblecombei* and therefore lower number of nymphs.

There was a significantly high positive correlation between the mean abundance of the commensal and the parasitism fauna's functional groups and the mean number of eggs of *G. brimblecombei* per leaf (N=6). The Spearman correlation coefficient was 0.829 (p-value = 0.021) for the commensals, 0.794 (p-value = 0.030) for the parasitoids. The predators fauna's functional group did not have a significantly high positive correlation with the mean number of eggs of G. brimblecombei per leaf (N=6), with a Spearman correlation coefficient of 0.371 (p-value = 0.234) for the predators.

3.5.2 – *Ctenarytaina spatulata*

In total, we found 19 insect specimens associated with *C. spatulata.* These specimens included two different insect species, native to Europe and Portugal, the ant *Plagiolepis schmitzii* (Hymenoptera, Formicidae) and a hoverfly *Sphaerophoria scripta* Linnaeus (Diptera, Syrphidae) (Table 4).

Table 4 – List of the species of insect fauna found associated with *Ctenarytaina spatulata* during the weekly survey, ordered by their functional group, taxonomic position and origin.

Functional group	Order	Family	Species	Origin	N
Commensal	Hymenoptera	Formicidae	*Plagiolepis schmitzii*	Native	4
Predator	Diptera	Syrphidae	*Sphaerophoria scripta* L.	Native	12

No parasitoid of *C. spatulata* was found during the survey. The commensal ant species associated with *C. spatulata* was only found during the beginning of the study in January/February, while the syrphid was found during the whole study period (Figure 7).

3.5.3 *Ctenarytaina eucalypti*

In total, 38 insect specimens were found associated with *C. eucalypti* belonging to two species, a native predator *S. scripta* and an alien parasitoid *Psyllaephagus pilosus* Noyes (Table 5).

Table 5 – List of the species of insect fauna found associated with *Ctenarytaina eucalypti* during the weekly survey, ordered by their functional group, taxonomic position and origin.

Functional group	Order	Family	Species	Origin	N
Predator	Diptera	Syrphidae	*Sphaerophoria scripta*	Native	1
Parasitoid	Hymenoptera	Encyrtidae	*Psyllaephagus pillosus* Noyes	Alien	37

No commensal species were found associated with *C. eucalypti.* The only native predator was a syrphid species *Sphaerophoria scripta*, found only once during the studied period (Table 5).

The mean number of commensals and predators found associated was significantly different for each psyllid species. *G. brimblecombei* had the highest mean abundance of associated commensals and predators registered in the fauna study with a mean number of 2.81 specimens in each survey sampling (N=10) (p-value=0.001; df=33; Chi^2=21.549 against *C. spatulata;* and p-value=0.001; df=32; Chi^2=5.26 against *C. eucalypti*). *C. spatulata* had an intermediate mean abundance of associated commensals and predators registered in the fauna study with a mean number of 0.35 specimens in each survey sampling (N=10) and finally, *C. eucalypti* had the lowest mean abundance of associated commensals and predators registered, of 0.07 specimens in each survey sampling (N=10) (p-value=0.025; df=53; Chi^2= 25.26, between *C. eucalypti* and *C. spatulata)*.

The maximum number of commensals and predators associated with *G. brimblecombei* were found in June and decreased thereafter (Figure 7). Regarding *C. spatulata* maximum numbers were found in March (Figure 8). Only one specimen was found for *C. eucalypti* (Figure 9).

Similarly the differences among species on the richness (r_s) of commensals and predators was significant (Chi^2 =18.471, df = 2, p<0.001). With values significantly higher for *G. brimblecombei* (r_s =14) in comparison with the *Cternarytaina* species. Only two species were observed associated with *C. spatulata* and only one with *C. eucalypti*.

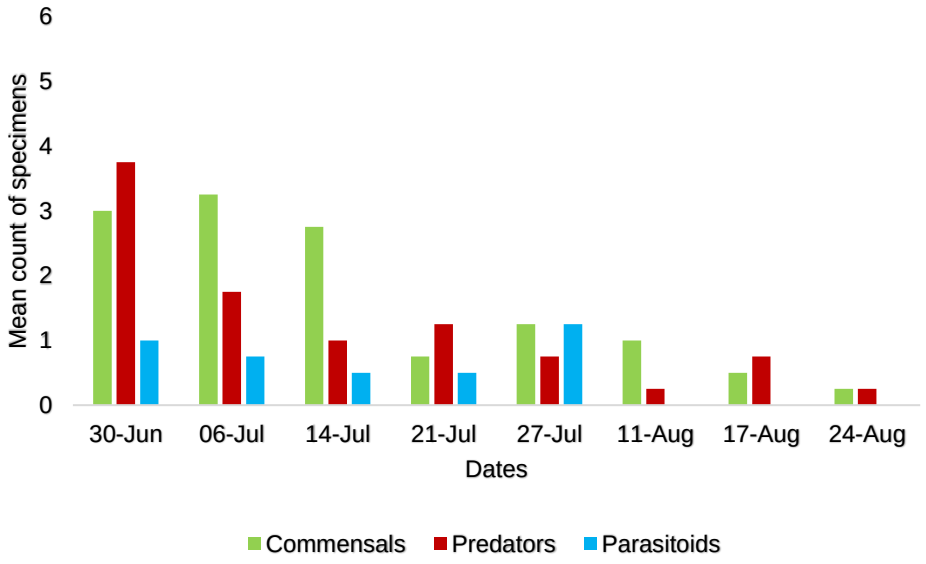

Figure 6 – Mean number of commensals, predators and parasitoids per sampling date found associated with *Glycaspis brimblecombei* on *Eucalyptus camaldulensis* in the two study sites, at Tapada da Ajuda, Lisbon, for each survey date of the weekly fauna survey done from 30/06/2015 to 24/09/2015 (N=10).

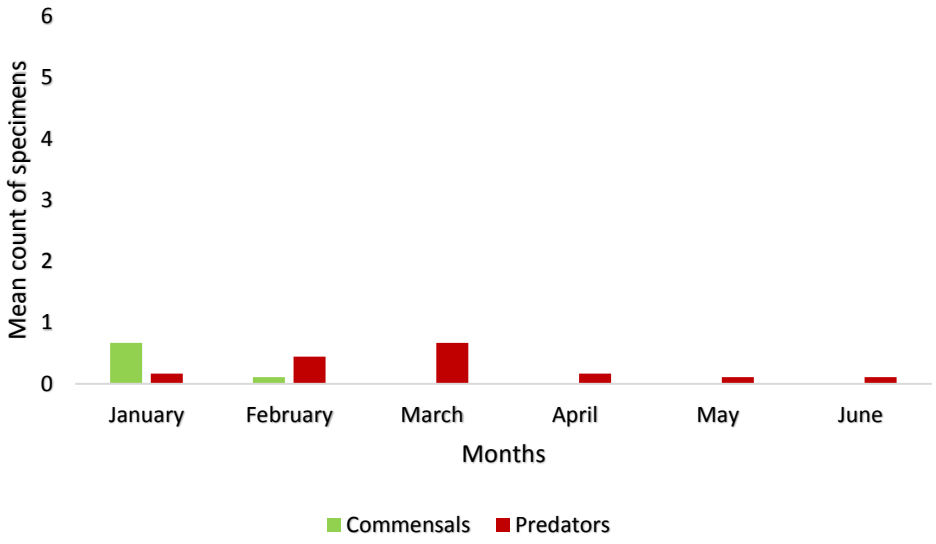

Figure 7 – Mean number of commensals and predators per sampling date found associated with *Ctenarytaina spatulata* on the *Eucalyptus camaldulensis* and *Eucalyptus rudis* in the two study sites, At Tapada da Ajuda, Lisbon, for each month of the weekly fauna survey done from 20/01/2016 to 30/06/2016 (N=10).

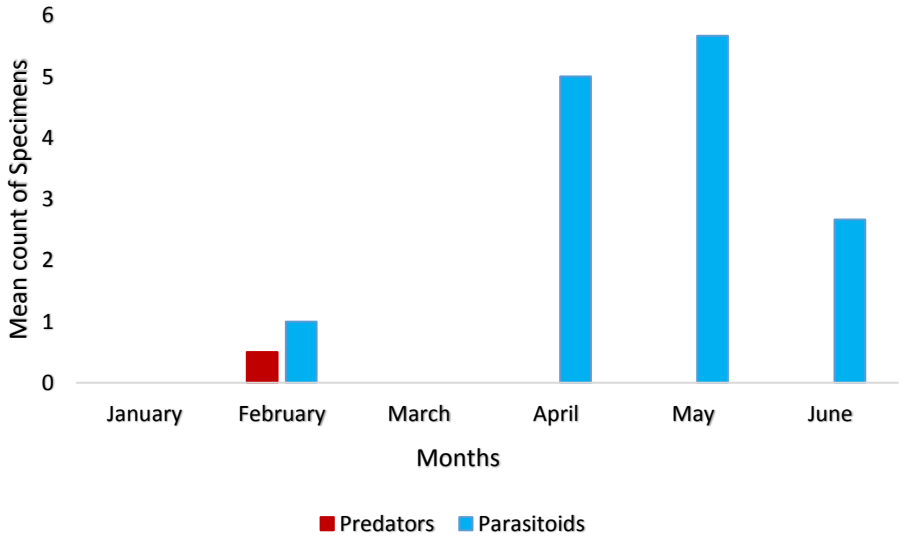

Figure 8 – Mean number of predators and parasitoids per sampling found associated with *Ctenarytaina eucalypti* on *Eucalyptus globulus* trees in the Arboreta study site, At Tapada da Ajuda, for each month during the weekly fauna survey done from 28/01/2016 to 30/06/2016 (N=10).

3.6 Honeydew

3.6.1 Collection values

The honeydew of the three studied psyllid species showed different properties and structure. The *G. brimblecombei* honeydew is very fluid and stored mostly under each nymph´s lerp or on the leaf, in the case of the adults, with a great accumulation of the honeydew in a single ball structure (Figure 9). The *C. spatulata* honeydew structure is similar to that of *G. brimblecombei*, but less fluid and without the production of lerps. In this case, the honeydew accumulates on the leaves, being connected to the psyllid by a string structure (Figure 10 and 11). Finally, the *C. eucalypti* honeydew is even less fluid and so more solid. Most of it is part of a dense structure which surrounds the colony of nymphs and the leaves, resembling a spider´s net and making the honeydew look like cotton (Figure 12).

The three psyllid species also excreted different amounts of honeydew. The amount of honeydew accumulated varied much among leaves and colonies, according to the number of nymphs in each leaf and nymphal stages. Therefore, the quantity of honeydew collected is merely indicative. For *G. brimblecombei*, we estimated the honeydew mean weight per leaf. We collected 250 mg of honeydew from 150 eucalypt leaves, which correspond to 1.67 mg of honeydew per leaf. For *C. eucalypti,* we collected 23.1 mg of honeydew from 20 eucalypt, corresponding to 1.1 mg per leaf. Finally, for *C. spatulata* we collected 22 mg of honeydew from 24 eucalypt leaves, corresponding to a 0.91 mg of honeydew per leaf. Considering that the mean number of infested leaves per branch corresponded to 12 during the higher activity period of *G. brimblecombei,* and of four leaves per branch for *C. spatulata* and *C. eucalypti*, we estimated the mean amount of honeydew produced per infested branch of 20mg for *G. brimblecombei*, 4.4mg for *C. eucalypti,* and 3.6mg for *C. spatulata*.

Figure 9 – A *Eucalyptus camaldulensis* tree leaf infested by *Glycaspis brimblecombei* with a closer look of two lerps of *Glycaspis brimblecombei* nymphs and the adult stage of the psyllid next to them (photo by Vera Zina).

Figure 10 – Colony of *Ctenarytaina spatulata* on an *E. camaldulensis* leaf shoot, with a high amount of produced honeydew (photo by André Garcia).

Figure 11 – A single honeydew droplet from a *Ctenarytaina spatulata* nymph on a *Eucalyptus camaldulensis* leaf (photo by Pedro Nunes).

Figure 12 – *Ctenarytaina eucalypti* colonies on a leaf shoot (1) and on a leaf (2) of *E. globulus*, with the presence of honeydew involving the colony with its net like structure from multiple layers of honeydew strings (Both photos by André Garcia).

3.6.2 Honeydew sugar components analysis

On the four samples of *G. brimblecombei* honeydew, two obtained from the lerps, and two from the liquid honeydew collected, the main sugar compounds were similar (Table 6).The most representative sugar of the four samples was the monosaccharide fructose. The higher levels of fructose in the lerps in comparison with the honeydew collected from the infested leaves of *E. camaldulensis,* and especially in the one from *E. tereticornis,* are of notice (Table 6). The other more representative sugars in the four samples are glucose and an unidentified peak. For this unidentified peak we did not have the standard to be able to identify it, but it's positioning reveals it may be the dissacharide isomaltose, being both of these sugars present in relatively high levels in all of the honeydew and lerp samples (Table 6). It is aim of future studies to identify these.

Other present sugars in all of the samples were the disaccharides turanose, maltose and melebiose and the trisaccharides melezitose and erlose. All of these sugars were found with similar levels of concentration in all four honeydew and lerp samples, except turanose, which was found in significantly higher levels in the lerp samples (Table 6). The remaining sugars are rhamnose, xylose, arabinose, sucrose and trehalose. From these, the rhamnose sugar levels differed significantly between lerp and liquid honeydew samples, being present in very low concentration levels in the lerp samples (<LQ) (Table 6). Sucrose concentration levels in all of the samples was low (<LQ), except in the lerps A sample, where the concentration levels were low but quantifiable, being barely higher than LQ (Table 6). In the opposite way, trehalose was present in intermediate concentration levels in all of the samples but the lerps A sample, where it´s concentration levels were too low to be quantified (<LQ) (Table 6). Arabinose sugar was only found in quantifiable concentration levels in the sample of honeydew of *E. tereticornis,* thought at a very low level of concentration, being its concentration levels too low to be

quantified(<LQ) in the remaining three samples. Finally xylose was not quantified in any of the four samples, with sugar concentration levels too low for its quantification (<LQ) (Table 6).

We had two different honeydew samples, collected from two host tree species. The two different honeydew samples had overall similar sugar concentration levels, with the same sugars having high or low levels of concentration. Fructose, which was the most representing sugar in both samples, was significantly higher in the *E. camaldulensis* honeydew over the honeydew on *E. tereticornis* (Table 6). Comparing Comparing with each other, we noticed similar values for most sugars. Still, for some other sugars, there were differences in the sugar concentration levels across samples. The *E. tereticornis* samples had higher levels of arabinose than the E. camaldulensis samples. This was because this sugar´s concentration level was only high enough in the *E. tereticornis* samples to allow its quantification (>LQ) (Table 6). The *E. camaldulensis* samples had higher levels of fructose and trehalose sugars (Table 6).

Table 6 – Identification and quantification of the individual sugar components in the solution samples, being represented as the concentration level of each sugar component of the total soluble solids of the samples of *G. brimblecombei* (g/100g of TSS).

Sugar	**Lerps A**	**Lerps B**	***E. camaldulensis***	***E. tereticornis***	**ANOVA**	
					F (df)	**p-value**
Rhamnose	<LQ	<LQ	0.67±0.46	0.86±0.06	0.322 (1,8)	0.586
Xylose	<LQ	<LQ	<LQ	<LQ	-	-
Arabinose	<LQ	<LQ	<LQ	0.15±0.06	-	-
Fructose	$15.35\pm0.81_{(a)}$	$9.56\pm0.55_{(b)}$	$8.08\pm0.69_{(c)}$	$4.93\pm0.11_{(d)}$	94.106 (3, 10)	0.001
Glucose	2.43±0.011	1.87±0.12	1.76±0.54	2.18±0.18	1.394 (3,10)	0.301
Sucrose	0.67	<LQ	<LQ	<LQ	-	-
Turanose	$1.56\pm0.04_{(a)}$	$1.02\pm0.08_{(b)}$	$0.70\pm0.10_{(c)}$	$0.65\pm0.02_{(c)}$	58.891 (3,10)	0.001
Maltose	1.66±0.08	1.65±0.05	1.80±0.14	0.98±0.06	0.544 (3,10)	0.663
Melebiose	0.98±0.03	0.89±0.10	0.60±0.36	0.51±0.03	1.346 (3,10)	0.314
Trehalose	<LQ	$1.19\pm0.01_{(a)}$	$1.00\pm0.11_{(a)}$	$0.71\pm0.18_{(b)}$	9.321 (2,9)	0.006
Melezitose	1.25±0.15	0.98±0.05	0.93±0.33	0.78±0.04	0.227 (3,10)	0.875
Erlose	$2.35\pm0.13_{(a)}$	$1.06\pm0.07_{(c)}$	$1.61\pm0.20_{(b)}$	$1.48\pm0.00_{(b)}$	19.675 (3,10)	0.001
Peak not identified*	2.12±0.20	1.48±0.01	1.81±0.20	1.80±0.03	-	-

*The unidentified peak is thought to be isomaltose, being a disaccharide sugar. This peak concentration levels was calculated by comparing their peak area with the sum of the peak area of the total sugar present in each sample. It is not a real value but an approach to be possible to compare with the other sugars.

Primarily due to the differences in the main sugar, fructose, the sugar composition representation of every sample is mostly of monosaccharides, being highest in the lerps A samples and being much lower in the honeydew samples of *E. tereticornis,* due to the much lower fructose levels. Still monossacharides are the most representative group type sugar for all the samples (Table 7).

Table 7 – Each sugar group type total representation in the sugar composition of each of the sampled solutions of *G. brimblecombei* (g/100g of TSS).

Honeydew origin	Monosaccharides	Disaccharides*	Trisaccharides
Lerps A	17.79	6.65	3.60
Lerps B	11.43	6.42	2.04
E. camaldulensis	10.51	5.52	2.86
E. tereticornis	8.12	4.66	2.26

* These values contain the calculated approach value for the unidentified peak.

4. Discussion

During a one year survey we studied the seasonal dynamics of three psyllid species of eucalypts in two sites, at Lisbon. For the three different psyllid species studied, the climatic conditions are clearly very impacting factors, in particular temperature and precipitation impacting factors, as reported by Boavida *et al*. (2016).

Glycaspis brimblecombei was active during the hot and dry periods of the year. The *G. brimblecombei* population made its appearance in the end of May, with a very relevant population increase, reaching its peak in June (Figure 1).

The appearance and quick rise of *G. brimblecombei* population is associated with the ending of precipitation, in the second half of May of 2016 (Figure 2). With the end of the rainfall there was also a significant rise of temperature associated with the population peak in June, where the temperature reached higher values (Figure 1). The afterwards fall of the species population in July and August is associated with the continued increase of temperature and lack of rain. These meteorological conditions clearly contributed for the decline of the population. This same effect on *G. brimblecombei* populations was also observed by Margiotta *et al*. (2017) in Italy in similar meteorological conditions.

Finally, the species activity continued to decrease in September and October, and was no longer detected in November. This may be related with rain in September and October (Figure 2), and/or the decrease in temperature. While the temperature conditions did play a role for the population numbers, the most crucial climatic factor for *G. brimblecombei* population seemed to be the absence of rainfall. This is not surprising, since it is known that the rainfall tends to be a negatively high impact factor for small leaf tending insects, including psyllids, which are very easily washed away from the leaves by the rain. We verified that *G. brimblecombei* population outside its active period still remains present in eucalypts through the rest of the year, but in very low numbers. The same observation was also reported in previous studies carried out in Portugal and Italy (Boavida *et al*., 2016; Margiotta *et al*., 2017).

The two *Ctenarytaina* species, *C. spatulata* and *C. eucalypti,* had similar seasonal population activities distributed during winter and spring, and thus differing from *G. brimblecombei*. The presence of both species during the cold and rainy months may suggest lower optimal temperatures for development and survival in comparison with those of *G. brimblecombei*. In fact, optimal temperature in laboratory conditions was found to be about 26ºC for *G. brimblecombei* (Firmino, 2004), agreeing with its summer distribution in our study, whereas the two *Ctenarytaina* species developed when mean temperatures varied between 13ºC in February and 17.6ºC in May.

The *Ctenarytaina* species also showed less vulnerability towards rain falling which may be associated to its predominant use of leaf branches with shoots, unlike *G. brimblecombei*, with its closed structure providing sheltering for the nymphs, which should reduce the dragging and falling of the psyllid nymphs from the rain. On the other hand, the protective lerp of *G. brimblecombei* might be easily washed by rain, removing its protective cover and implying costly efforts in producing new lerp. These two factors may be responsible for *G. brimblecombei* being clearly less resilient to the rain`s washing effect in comparison with *Ctenarytaina* species.

Our study also shows differences on the *G. brimblecombei* and *C. spatulata* psyllids population's activity in the two different sites. The fact that in Eira Velha the trees were mature, more than 30 years old, whereas in the Arboreta, trees were young, less than 9 year old, may justify the higher infestation values of the two psyllid species in Arboreta. This result suggests a higher susceptibility of younger plantations to these insect pests. Related to this, we found that even though *G. brimblecombei* does infest all the leaves of eucalypt branches, it showed a preference for the younger and medium aged leaves over the older ones, from the basal position of the branch. These younger aged leaves, which should be more abundant in younger trees, are sink organs, in which psyllids may feed on a nutrient richer sap.

Still, for *G. brimblecombei*, the *E. camaldulensis* trees in Eira Velha were very highly infested and differences among sites were minor, whereas for *C. spatulata* the leaf infestations values were much lower in Eira velha in comparison with the Arboreta. These differences among species might be justified by the higher preference and dependence of *C. spatulata* from young shoots (Valente *et al*., 2004), possibly produced in higher proportion on young trees.

Regarding differences between host plant species, we could not find differences on the infestation level of *C. spatulata* between *E. camaldulensis* and *E. rudis*. The fact that these two species are phylogeneticaly close, both of them from section Exertaria, may justify the similar performance of the psyllid on both host species. A similar relationship was observed in eucalyptus gall wasps (Branco *et al*., 2014).

The two *Cternarytaina* species had very low commensal and predator associated fauna numbers and the fauna was not diverse, having ants as the only commensal species and only one native predator species. Oppositely, for *G. brimblecombei* we registered 11 different commensal and three predator species associated with this psyllid. Curiously, the unique ant associated with *C. spatulata*, *P. schmitzii,* was also the dominant ant species associated with *G. brimblecombei.*

The non-favoring meteorological registered during the period in which the two *Cternarytaina* species were active may have had a negative effect on the associated fauna. Also, the two

Cternarytaina species do have a different ecological niche in relation to *G. brimblecombei*, with a restricted use of only young developing leaves. The broader niche of *G. brimblecombei*, developing on different types of leaves, with a wider host range and producing a much higher amount of honeydew, should facilitate associations with more diverse fauna.

The commensal interactions with the native fauna were the most common. In total, 11 different species had a confirmed commensal interaction with *G. brimblecombei*. All the commensal interactions were associated with honeydew consumption. Some of these species were already reported as honeydew consumers (Wäckers et al. 2008). These number of species should still be lower than reality, not only because, most likely, not all honeydew feeding insects were found but also because other known honeydew-feeding animals, such as birds (Paton, 1980; Douglas, 2006), were not studied.

Three predators were found associated with *G. brimblecombei*, *i.e. Anthocoris nemoralis* Fabricius, *Chrysoperla carnea* Stephens and *Coenosia attenuata* Stein. Another predatory species, *Sphaerophoria scripta* Linnaeus was found associated with the two *Ctenaryrtaina* species.

Anthocoris nemoralis has been reported as a predator of psyllids and specifically of *G. brimblecombei* (Hagen and Dreistadt, 1990; Brennan *et al*., 1999; Valente and Hodkinson, 2009; Laudonia *et al*., 2014). This predator might thus be a potential biological control agent for the psyllid species population whose efficacy should be further investigated. Still, the number of specimens of this species was low, which might be due to the harsh climatic conditions of 2015, with long periods of high temperature and lack of humidity (Figure 2). The neuropteran insect order in general has been mentioned to predate over psyllid nymphs in many studies (Brennan *et al*., 1999; Michaud *et al*., 2002; Laudonia *et al*., 2014) and so they can be considered as a potential biological agent from the native fauna. In Portugal, the predominant neuropteran species is *Chrysoperla carnea*, which is the species found associated with *G. brimblecombei,* predating its nymphs.

We further found a predatory fly, *Coenosia attenuata* that has been reported predating on midflight *G. brimblecombei* adults (Figueiredo *et al*. 2013). Thought its abundance in the eucalypts was low it should still be taken into consideration as an additional factor for the control of *G. brimblecombei* population.

The syrphid larvae, just like the neuropteran larvae, are ferocious generalist predators that have been reported to consume sap-suckers, including psyllids (Figo and Silva, 1977; Michaud, 2002; Laudonia *et al*., 2014). We only found specimens of these and eggs associated with *Ctenarytaina*. The syrphid species found associated with *Ctenarytaina* species *S. scripta* has already been referenced in a previous study as a predator of *C. eucalypti,* in *E. globulus,*

in Portugal (Figo and Silva, 1977). The much higher number of specimens of this syrphid found associated with *C. spatulata* in comparison with *C. eucalypti* was most likely due to the higher densities of *C. spatulata* in the Arboreta site.

Previous studies referred other predators of *G. brimblecombei*, such as spiders and ladybirds (Laudonia *et al*., 2014). These two taxa were found in the Arboreta during the period of activity of *G. brimblecombei,* but were never found predating on the psyllids and so were not accounted for in our study. However, they should be noted as potential predators due to their presence in the leaves and already having been reported in other works as predators (Michaud, 2002). Ladybirds could also be commensals exploiting honeydew (Wäckers et al, 2008).

The mirid *Campyloneura virgula* Fieber, a generalist predator, was also found in eucalypt leaves with *G. brimblecombei*. Even though not mentioned in any past work, the species does have a potential to predate over the nymphs of the psyllids. Still, predatory activity was not confirmed and so it was not accounted for in our study.

These predation and commensal interactions demonstrate that the presence of the sap-sucking species can positively affect the native fauna by offering to these usually generalist species new options to their diet. To study the effect of the presence and population variation of the introduced invasive psyllid species on the local native species and how it affects the biodiversity of the local fauna, either beneficial or negative, is thus particularly relevant.

The two parasitoid species, *i.e. P. bliteus* found associated with *G. brimblecombei,* and *P. pillosus*, which was associated with *C. eucalypti*, were both previously reported in Portugal. These parasitoids are native to Australia and were fortuitously introduced in Portugal (Figo and Silva, 1977; Dahri *et al*. 2014). *P. bliteus* was introduced in California, in 2000, for the biological control of *G. brimblecombei* (Dahlsten *et al*., 2005) and from there it was introduced with its host in other regions (Boavida *et al*., 2016). *P. pillosus* was introduced in France from where it dispersed to other European regions (Hodkinson, 1999). For *C. spatulata*, we found no parasitoids. No parasitoids of this psyllid species have been reported outside its native range.

The number of eggs of *G. brimblecombei* population, showed a significant positive correlation with the abundance of the associated fauna. Thought it was not significantly between the predators and the number of eggs, it was shown by the presence of predators, that they were attracted by the presence of the psyllids to the eucalypts. This results show a consistent relationship between these fauna elements and the food resources provided by the psyllids, either their honeydew, as prey or both.

Regarding sugar composition of psyllid honeydew we were able to determine the presence of many compounds, mostly monosaccharides and disaccharides. The sugars, melezitose,

glucose, fructose and turanose that we found in all the four honeydew and lerp samples had already been reported, but had not been quantified, in the honeydew of *Eriococcus coriaceus* Maskell and *Eucalyptoloma maidenii* Froggatt (Basden, 1970).

Most of the sugars that we studied had similar values in the composition of the honeydew and lerp samples, with the fructose levels being the highest in all four samples. Still, we found differences between the lerps and honeydew samples, which can be related to differences on these two products. For example, the fructose and turanose levels were both overall higher in the lerp samples than in the honeydew samples, while rhamnose levels were higher in the honeydew samples than in the lerp samples. These sugar composition differences between lerps and honeydew can contribute to the different products differences, thought much more work will have to be done in this matter.

Fischer and Shingleton (2001) investigated how different host species contribute to differences in aphid honeydew composition. However, as far as we know, this issue was not studied in psyllids. In the case of *G. brimblecombei,* we did not find clear differences between the honeydew samples from different tree species, with the only significant difference being the higher level of fructose in the *E. camaldulensis* honeydew samples. This similarity between host species can be related to the fact that the two eucalypt species are closely related and both are highly susceptible species to *G. brimblecombei* (Wilcken et al. 2003).

For future studies, it would be interesting to compare the honeydew composition among the three studied psyllid species of eucalypt. Due to the low amount of honeydew, the use of different methods of sugar composition analysis requiring lower sample amounts, like the Raman spectroscopy which seems like a promising recent technique which could be an alternative (Ruoff, 2006).

5. Conclusion

With this work we were able to determine the different seasonal activity of the three psyllid species in eucalypts in Lisbon, Portugal. While *G. brimblecombei* was active in the hot and dry months, the two *Ctenarytaina* species were present in the colder and wetter months. Additionally, whereas *G. brimblecombei* occurred on all types of leaves, the two *Ctenarytaina* species preferred young developing leaves, having been verified that the younger leaves and younger trees of eucalypts are more susceptible to these psyllid species.

The abundance of commensal and predator native fauna associated with *G. brimblecombei* was much higher than the one associated with *Ctenarytaina* species. It was also verified that the abundance of these associated native fauna specimens on the eucalypt leaves was positively correlated with the psyllid population of *G. brimblecombei*. Therefore there is a relevant positive effect of the presence of honeydew producing invasive species on the insect native fauna on the eucalypts. We found a high diversity of commensal species associated with the psyllids, comprising 11 species distributed by 3 orders. This work serves as a reference for future studies of commensal interactions between introduced honeydew producing insects and the native insect fauna on eucalypts.

The most important predator species found associated with the psyllids were *A. nemoralis* and *C. carnea* for *G. brimblecombei,* and *S. scripta* for *C. eucalypti* and *C. spatulata*. Our results provide a better understanding of the three eucalypt psyllid species dynamics and the identification and characterization of potential biological control agents of the psyllid populations. The monitoring and studying of these agents impact in the psyllids populations, alongside the understanding of the species activity dynamics, should prove important for future eucalypt plantation management and control of the psyllids species in Portugal.

Finally the honeydew characteristics and quantity differed among the three psyllid species. In the case of *G. brimblecombei,* the lerps had higher levels of fructose in comparison with liquid honeydew. The honeydew of *G. brimblecombei* when feeding on *E. camaldulensis* was not significantly different from that produced on *E. tereticornis*. These honeydew and lerp studies should serve as a guideline for future studies, in which it would be interesting to further research the effect of honeydew and lerp sugar differences on the fauna attracted to the psyllids.

References

Alves, A.M., Pereira, J.S., and Silva, J.M.N. (2007). A Introdução e a Expansão do Eucalipto em Portugal. In: Alves, A.M., Pereira, J.S., Silva, J.M.N. (eds) O Eucaliptal em Portugal. Impactes Ambientais e Investigação Científica. ISA Press, Lisbon, 13–24.

Basden, R. (1970). A note on the composition of the lerp and honeydew of *Eucalyptolyma maidenii* Froggatt. In Proceedings of the Linnean Society of New South Wales, 95(1), 9-10.

Boavida, C., Garcia, A., and Branco, M. (2016). How effective is *Psyllaephagus bliteus* (Hymenoptera: Encyrtidae) in controlling *Glycaspis brimblecombei* (Hemiptera: Psylloidea)?. Biological Control, 99, 1-7.

Bogdanov, S., Martin, P., and Lullmann, C. (2002). Harmonised methods of the international honey commission. Swiss Bee Research Centre, FAM, Liebefeld.

Bouchard, Y., and Cloutier, C. (1984). Honeydew as a source of host-searching kairomones for the aphid parasitoid Aphidius nigripes (Hymenoptera: Aphidiidae). Canadian Journal of Zoology, 62(8), 1513-1520.

Branco, M., Dhahri, S., Santos, M., and Jamaa, M.L.B. (2014). Biological control reduces herbivore's host range. Biological Control, 69, 59-64.

Brennan, E.B., Gill, R.J., Hrusa, G.F., and Weinbaum, S.A. (1999). First record of *Glycaspis brimblecombei* (Moore) (Homoptera: Psyllidae) in North America: initial observations and predator associations of a potentially serious new pest of eucalyptus in California. Pan-Pacific Entomologist, 75(1), 55-57.

Buckley, R. (1987). Ant-plant-homopteran interactions. Advances in Ecological Research, 16, 53-85.

Burckhardt, D. (1994). Psylloid pests of temperate and subtropical crop and ornamental plants (Hemiptera, Psylloidea): a review. Trends in Agricultural Sciences, Entomology, 2, 173-186.

Burgiel, S., Foote, G., Orellana, M., and Perrault, A. (2006). Invasive alien species and trade: integrating prevention measures and international trade rules. The Center for International Environmental Law and Defenders of Wildlife, Washington, DC, 66-74.

Chinery, M. (1993). A Field Guide to the insects of Britain and Northern Europe. Third edition, HarperCollins.

Choate, P.M. (1999). Introduction to the identification of beetles (Coleoptera). Dichotomous Keys to some Families of Florida Coleoptera. Wood, Berks. Entomo Mont Mag, 102, 156-162.

Choate, P. M. (2010). "Identification Key to the Principal Families of Florida Heteroptera."

Coppen, J.J.W. (2002). Eucalyptus - The Genus *Eucalyptus*. Taylor and Francis, New York.

Dahlsten, D., K. Daane, T. Paine, K. Sime, A. Lawson, D. Rowney and W. Roltsch (2005). "Imported parasitic wasp helps control red gum lerp psyllid." California Agriculture 59(4), 229-235.

Dhahri, S., Jamaa, M.L., Garcia, A., Boavida, C. and Branco, M. (2014). Presença do *Glycaspis brimblecombei* e do parasitoide *Psyllaephagus bliteus* na Tunísia e em Portugal. Silva Lusitana, 22(1), 99-105.

Douglas, A.E. (2006). Phloem-sap feeding by animals: problems and solutions. Journal of Experimental Botany, 57(4), 747-754.

Figo, M.L., and Silva, L.P (1977) A *Cternarytaina eucalypti* Maskell, Praga dos eucaliptos. Secretaria de Estado das Florestas. Direcção-Geral dos Recursos Florestais, 4-34.

Figueiredo, E., Nunes. R., Martins, J., Franco, J.C., Garcia, A., Rebelo, T., Paulo, O., Seabra, S., and Mateus, C. (2013). Has tiger-fly a role in biological control of protected crops?. 4th Int. Symp. Biological Control of Arthropods, Púcon, Chile, 4-8.

Firmino, D.C. (2004). Biologia do psilídeo-de-concha *Glycaspis brimblecombei* Moore (Hemiptera: Psyllidae) em diferentes espécies de eucalipto e em diferentes temperaturas. 49f. Dissertação de Mestrado em Proteção de Plantas. Faculdade de Ciências Agronômicas, Universidade Estadual Paulista.

Firmino, D.C., Wilcken, C.F., Oliveira, N.C.D., and Matos, C.A.O.D. (2009). Red gum lerp psyllid *Glycaspis brimblecombei* Moore (Hemiptera, Psylidae) biology in *Eucalyptus* spp. Revista Brasileira de Entomologia, 53(1), 144-146.

Fischer, M.K., and Shingleton, A.W. (2001). Host plant and ants influence the honeydew sugar composition of aphids. Functional Ecology, 15(4), 544-550.

Gill, R.J. (1998). New state records: Redgum lerp psyllid, *Glycaspis brimblecombei*. California Plant Pest and Disease Report, 17(1-3), 7-8.

Goulet, H., and Huber, J.T., (1993). Hymenoptera of the World: An Identification Guide to Families. Centre for Land and Biological Resources, Ontario, USA.

Hagen, K.S., and Dreistadt, S.H. (1990). First California record for *Anthocoris nemoralis* (Fabr.)(Hemiptera: Anthocoridae), a predator important in the biological control of psyllids (Homoptera: Psyllidae). Pan-Pacific Entomologist, 66(4), 323-324.

Hodkinson, I.D. (1974). The biology of the Psylloidea (Homoptera): a review. Bulletin of Entomological Research, 64(02), 325-338.

Hodkinson, I.D. (1999). Biocontrol of eucalyptus psyllid Ctenarytaina eucalypti by the Australian parasitoid *Psyllaephagus pilosus*: a review of current programmes and their success. Biocontrol News and Information, *20*(4), 129N-134N.

Hollis, D. (2004). Australian Psylloidea. Jumping plant lice and lerp insects. Canberra (Australia): Australian Government, Australian Biological Resource Study.

Hurley, B.P., Garnas, J., Wingfield, M.J., Branco, M., Richardson, D.M., and Slippers, B. (2016). Increasing numbers and intercontinental spread of invasive insects on eucalypts. Biological Invasions, 18(4), 921-933.

ICNF – Instituto de Conservação da Natureza e Florestas, (2013). IFN6 – Áreas dos usos do solo e das espécies florestais de Portugal continental em 1995, 2005 e 2010. Instituto da Conservação da Natureza e das Florestas. Lisboa, 34pp.

Kenis, M., Auger-Rozenberg, M.A., Roques, A., Timms, L., Péré, C., Cock, M.J. Matthew., and Lopez-Vaamonde, C. (2008). Ecological effects of invasive alien insects. In Ecological impacts of non-native invertebrates and fungi on terrestrial ecosystems. Biological invasions, 11(1) Springer, Netherlands, p. 21-45.

Laudonia, S., Margiotta, M., and Sasso, R. (2014). Seasonal occurrence and adaptation of the exotic *Glycaspis brimblecombei* Moore (Hemiptera: Aphalaridae) in Italy. Journal of Natural History, 48(11-12), 675-689.

Margiotta, M., Bella, S., Buffa, F., Caleca, V., Floris, L., Giorno, V., Lo Verde, G., Rapisarda, C., Sasso, R., Suma, P., Tortorici, F., and Laudonia, S. (2017). Modeling enviromental influences in the *Psyllaephagus bliteus* (Hymenoptera: Encyrtidae) – *Glycaspis brimblecombei* (Hemiptera: Aphalaridae) parasitoid – host system. Journal of Economic Entomology, advance access, 1-11. Doi: https://doi.org/10.1093/jee/tow253.

Mansfield, S. (2016). New Communities on Eucalypts Grown Outside Australia. *Frontiers in Plant Science*, *7*.

Meiracker, R.A.F. van den, Hammond, W.N.O., and van Alphen, J.J.M. (1990) The role of kairomone in prey finding by *Diomus* sp. and *Exochomus* sp., two coccinellid predators of the cassava mealybug, *Phenacoccus manihoti*. Entomologia Experimentalis et Applicata, 56, 209J217.

Chinery, M. (1973). A Field Guide to the insects of Britain and Northern Europe. Third edition, Collins, London.

Michaud, J.P. (2002). Biological control of Asian citrus psyllid, Diaphorina citri (Hemiptera: Psyllidae) in Florida: a preliminary report. Entomological News, 113(3), 216-216.

Paine, T.D., Millar, J.G., and Daane, K.M. (2010). Accumulation of pest insects on eucalyptus in California: random process or smoking gun. Journal of Economic Entomology, 103(6), 1943-1949.

Paton, D.C. (1980). The importance of manna, honeydew and lerp in the diets of honeyeaters. Emu, 80(4), 213-226.

Pimentel, D., Lach, L., Zuniga, R., and Morrison, D. (2000). Environmental and economic costs of non-indigenous species in the United States. BioScience, 50(1), 53-65.

Queiroz, D.L., Burckhardt, D. and Majer, J. (2012) Integrated pest management of eucalypt psyllids (Insecta, Hemiptera, Psylloidea). Integrated Pest Management and Pest Control – Current and Future Tactics (ed. by Larramendy, M. L. and Soloneski, S.), p. 385–412. InTech, Croatia.

Ruoff, K. (2006). Authentication of the botanical origin of honey (Doctoral dissertation). Swiss Federal Institute of Tecnology, Zurich.

Santana, D.L,Q., Andrade F.M., Bellote A.F.J. and Grigoletti A.J. (1999) Associação de *Ctenarytaina spatulata* e de teores de Magnésio foliar com a seca dos ponteiros de *Eucayptus grandis* (in Portuguese). Bol Pesq Fl 39:41–49.

Santana, D.L.Q. (2003). *Ctenarytaina spatulata* Taylor, 1997 (Hemiptera: Psyllidae): morfologia, biologia, dinâmica, resistência e danos em *Eucalyptus grandis* Hill. ex Maiden.

Tese de Doutorado de Ciências Biológicas (Entomologia). Universidade Federal do Paraná. Curitiba, 123p.

Santana, D.L., Burckhardt, D. (2007). Introduced Eucalyptus psyllids in Brazil. Journal of Forest Research, 12(5), 337-344.

Santana, D.L., Zanol, K. M., Botosso, P.P.C., and Mattos, P.P. (2005). Danos causados por *Ctenarytaina spatulata* Taylor, 1977 (Hemiptera: Psyllidae) em *Eucalyptus grandis* Hill. Ex Maiden. Boletim de Pesquisa Florestal, 50, 11-24.

Santana, D.L., and Zanol, K.M. (2006). Biologia de *Ctenarytaina spatulata* Taylor, 1997 (Hemiptera, Psyllidae) em *Eucalyptus grandis* Hill ex Maiden. Acta Biologica Paranaense, 35, 47-62.

Scudder, G.G.E., and Cannings, R.A. (2006). The diptera families of British Columbia. Acessed at http://www.for.gov.bc.ca/hfd/library/FIA/2006/FSP_Y062001b.pdf [04/09/2015].

Sharma, A., Raman, A., Taylor, G. and Fletcher, M. (2013). Nymphal development and lerp construction of *Glycaspis* sp. (Hemiptera: Psylloidea) on *Eucalyptus sideroxylon* (Myrtaceae) in central-west New South Wales, Australia. Arthropod Structure and Development, 42(6), 551-564.

Stubbs, A.E., and Falk, S.J. (1983). British hoverflies. An illustrated identification guide. British Entomological and Natural History Society, London.

Taylor, K.L. (1997). A new Australian species of *Ctenarytaina* Ferris and Klyver (Hemiptera: Psyllidae: Spondyliaspidinae) established in three other countries. Australian Journal of Entomology, 36(2), 113-115.

Trabado, G.I, and Wilstenmann, D. (2008), Eucalyptus Universalis. Globally cultivated eucalypt forest map 2008 Version 1.0.1 in GIT forest consulting´s EUCALYPTOLOGICS: Information resources on eucalyptus cultivation worldwide. Viewed from http://git-forestry-blog.blogspot.com/2008/09/eucalyptus-global-map-2008-cultivated. [August 28th 2016].

Valente, C., Manta, A., and Vaz, A. (2004). First record of the Australian psyllid *Ctenarytaina spatulata* Taylor (Homoptera: Psyllidae) in Europe. Journal of Applied Entomology, 128(5), 369-370.

Valente, C., and Hodkinson, I. (2009). First record of the red gum lerp psyllid, *Glycaspis brimblecombei* Moore (Hem: Psyllidae), in Europe. Journal of Applied Entomology, 133(4), 315-317.

Wäckers, F.L., Van Rijn, P.C., Heimpel, G.E. (2008). Honeydew as a food source for natural enemies: making the best of a bad meal? Biological Control, 45(2), 176-184.

Wilcove, D.S., Rothstein, D., Dubow, J., Phillips, A., and Losos, E. (1998). Quantifying threats to imperiled species in the United States. BioScience, 48(8), 607-615.

Wilcken, C.F., Couto, E.D., Orlato, C., Ferreira Filho, P.J., and Firmino, D.C. (2003). Ocorrência do psilídeo-de-concha (*Glycaspis brimblecombei*) em florestas de eucalipto no Brasil. Circular Técnica IPEF, (201), 1-11.

Withers, T.M. (2001). Colonization of eucalypts in New Zealand by Australian insects. Austral Ecology, 26(5), 467-476.

Zhang, W., and Chen, B. (2011). Environment patterns and influential factors of biological invasions: a worldwide survey. Proceedings of the International Academy of Ecology and Environmental Sciences, 1(1), 1-14.